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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 267 317**
A1

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 86115797.2

(22) Date of filing: 13.11.86

(51) Int. Cl.⁴: G01N 33/68 , G01N 33/569 , G01N 33/543

(43) Date of publication of application:
18.05.88 Bulletin 88/20

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI NL SE

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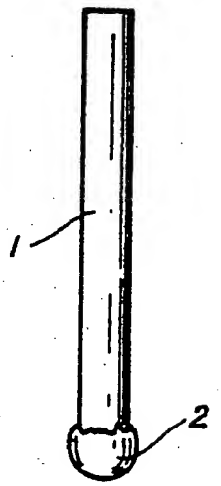
(54) Method for the detection of proteins and viruses.

(57) Proteins or viruses are detected by means of an immunoassay method in which an extended solid phase coated with antiprotein or antiviral antibody is employed to bind and remove proteins or virions from a specimen by forming an immuno-complex with antigens of said proteins or virions, a mobile solid phase comprising a dispersion of microspheres coated with the antiprotein or antiviral antibody is used to bind said microspheres to antigens associated with said immuno-complex, and the presence of bound microspheres is detected. The detection sensitivity is amplified by the ability to more readily detect the microspheres, which may be dyed or labelled. The extended solid phase advantageously may be in the form of a dipstick which can be easily contacted with the specimen. A virus detection kit provides the extended solid phase and mobile solid phases, each coated with antiprotein or antiviral antibodies.



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METHOD FOR THE DETECTION OF PROTEINS AND VIRUSES

The invention relates to the detection and/or the determination of proteins or viruses by an immunoassay method, to materials for such method, and to a protein or virus detection kit.

A number of methods are available in clinical virology which can be used to detect viruses or certain viral antigens (Ag). Infectivity assays depend upon biological amplification, i.e., the ability of viruses to multiply and provide adequate amounts for observation. In vitro methods include infection of cells for plaque formation or other readily observed cytopathic effects, whereas in vivo methods require an infection which causes the death of injected animals. These infectivity assays are accurate with respect to the presence of viable virions, but they do not directly provide viral specificity, and the procedures are tedious and time consuming.

As indicated in Diagnostic Virology, G. D. Hsiung, Third Edition (1982), Yale University Press, New Haven and London, pages 3-6, an alternative to these methods are immunoassays which depend on the de tection of a specific viral Ag by an antibody (Ab) which forms an immuno-complex with the Ag. Methods such as Immunofluorescence, Enzyme Immunoassay, and Radioimmunoassay are the three most well developed assays. They measure the presence of viral Ag, which can be the capsomere proteins of the virion, the neuraminidase and haemagglutinin "spikes" of the virion or the viral nucleic acids. These assay methods are highly specific, fast and sensitive relative to infectivity assays, but viral viability must still be verified by infectivity tests. Direct observation of virions using negative staining and an electron microscope or using immunoelectromicroscopy generally does not offer sufficient sensitivity, but has advantages of being faster than the assay methods and requiring a minimum of specimen preparation. The known assay methods have a number of limitations. Enzyme Immunoassay requires use of elaborate and costly chemistry. Radioimmunoassay involves steps requiring lengthy incubation times, and the difficulty of handling radioactive materials. Immunofluorescence methods are generally less sensitive. There is accordingly a need for a more sensitive, faster and economical method for the detection of viruses.

The method of choice, therefore, depends on the nature of the specimen, the need for rapid detection and/or high sensitivity, as well as the availability of detection instruments and reagents.

U.S. Patent No. 3,088,875 granted on May 7, 1963 to Roy T. Fisk, discloses tests for antigens or antibodies indicative of pathological conditions, employing polymeric styrene latex having particle size of 0.15 to 0.25 micron as a carrier for specific, known antibodies or antigens. The carrier is dyed, and the presence of unknown antibodies or antigens in a specimen is determined by visual observation of flocculation of the test reagent, due to formation of immune-complexes in the mixture on a glass slide.

The introduction of tracers or labels on to microscopic particles or strands of carrier material, and use of such labelled particles in immunoassays is described in U.S. Patent No. 3,853,987 granted on December 10, 1974 to William J. Dreyer, the disclosure of which is incorporated herein by reference.

In the present inventor's U.S. Patent 4,436,826 granted March 13, 1984 and U.S. Patent 4,454,233 granted June 12, 1984, antibody or antigen coupled microspheres having isolated tagging or labelling materials therein are disclosed for use in immunoassays. These applications also disclose use of a solid phase material which is employed for the separation of bound and free microspheres. A number of detection methods for the labelled microspheres are disclosed such as X-ray fluorescence.

The present invention provides a method and a kit for detection of viruses, virus proteins or other proteins. The viruses or proteins to be detected are herein referred to as "targets". An "antitarget" antibody is herein denoted by "Ab_v" which herein means, in the case of viruses an antiviral antibody, or in the case of proteins an antiprotein antibody.

According to the present invention there is provided a method for detection of target proteins or viruses in a specimen, wherein said specimen, treated to remove undesired components, is contacted with an extended solid phase support having conjugated thereon antitarget antibody (Ab_v) to form immuno-complexes with antigens characteristic of the proteins or viruses to be detected; the extended solid phase is separated from the specimen; said separated extended solid phase is contacted with a mobile solid phase consisting of dispersed microspheres having conjugated thereto said Ab_v to bind said microspheres to said immuno-complexes; the extended solid phase is separated from said mobile solid phase, and the presence of microspheres bound to said extended solid phase is detected, whereby the presence of proteins or viruses in said specimen is detected or determined.

Also, the invention provides a kit for detection of target proteins or viruses which comprises as individual components: (a) an extended solid phase having conjugated thereon antitarget antibody

(Ab_v) capable of forming immuno-complexes with antigens characteristic of the proteins or viruses to be detected; and (b) a mobile solid phase consisting of dispersed microspheres having said- Ab_v conjugated thereto.

In accordance with the present invention, a new method of immunoassay is disclosed which involves solid phase amplification and is particularly suitable for virus detection. The method of the invention uses an Ab-conjugated mobile solid phase which when bound to Ag can be measured by simple instruments with very high sensitivity.

Although the invention is applicable not only to viruses and virus proteins but also to other proteins for simplicity the following description will mainly refer to viruses. In this description what is stated about viruses also can be taken to refer to proteins.

Microspheres used in most embodiments of the present invention should have a size (longest linear dimension, or diameter if spherical) of substantially less than 0.5 µm. A size of about 0.3 µm or below is suitable in a number of circumstances. Preferably the size is about 0.25 µm or smaller, and more preferably it is 0.1 µm or smaller. Smaller sizes which are preferred are in the range of 0.1 µm to 0.008 µm.

A specimen which may comprise viruses of types to be detected is exposed to an extended solid phase component which is coated at least in one location with Ab_v which will form complexes with the antigens of the viruses to be detected. The extended solid phase is separated from the specimen, such as by washing the specimen off the extended solid phase, and the separated extended solid phase is then contacted with a mobile solid phase of dispersed microspheres including the same Ab_v thereon. If immuno-complexes of antigens of viruses to be detected ("target" viruses) have formed on the extended solid phase, the microspheres will be bound to such complexes.

The unbound microspheres of the mobile solid phase then are removed, such as by washing, and the extended solid phase is examined to determine the presence of microspheres bound to the extended solid phase. These may be visually detected in some cases, for example when the microspheres have been initially stained or dyed. Microscopic examination may be employed. The use of tracers or labels for the microspheres enables the use of other detection methods.

By this means, the presence or absence of bound microspheres enables detection of the presence or absence of the target viruses, and an evaluation of the quantity of bound microspheres enables determination of the quantity of viruses in the specimen, for example by comparison with standard results for the assay of known samples.

In the accompanying drawing:

Fig. 1 illustrates schematically an elevation of an embodiment of the extended solid phase of the invention in the form of a dipstick.

Fig. 2 illustrates schematically an elevation of another embodiment of the dipstick.

Fig. 3 illustrates schematically a sectional elevation of the extended solid phase of the invention in the form of a tubular container.

Fig. 4 is a schematic elevation of the extended solid phase of the invention in the form of a sphere for use with magnetically labelled microspheres.

Fig. 5 is a schematic representation of a detection device for use with the extended solid phase of Fig. 4.

The extended solid phase used in the present invention may be employed in a variety of forms or structures, it only being necessary for the solid phase to have a location where Ab_v is conjugated thereon, and for the solid phase with said Ab_v to be formed so as to enable contacting with the specimen and other materials used in the method of the invention. Thus, the extended solid phase is best formed in a way which enables simple manipulation for easy contact with the specimen and other reagents. For this purpose, the extended solid phase may form at least part of a dipstick, syringe, tube or container. The specimen and other reagents can be drawn in and ejected from a syringe, caused to flow through a tube, or deposited in a container such as a test tube shaped container. In such devices, the extended solid phase can form the whole of the device, or part of it, where, in the case of a syringe, tube or container, the part formed of the extended solid phase will at least be exposed at the inside of the device to permit contact with specimen and reagents. Preferably, Ab_v is concentrated at one location of the extended phase, to be exposed to the specimen.

The most preferred form of the extended solid phase is a dipstick. In such a dipstick, it is further preferred that the extended solid phase should be included at at least one end, and that the Ab_v conjugated on the extended solid phase should be concentrated at the end of the dipstick. The extended solid phase can however comprise the entire dipstick, with the Ab_v concentrated at one end, or in more than one location.

In Fig. 1 a dipstick is shown, which is entirely formed from the extended solid phase 1, at one end of which has been conjugated a coating 2 of Ab_v. Fig. 2 illustrates another dipstick embodiment made of the extended solid phase 1 one end of which is adhered to a body portion 3. A coating 2 of Ab_v is conjugated to the extended solid phase. In another embodiment shown in Fig. 3, the extended solid phase entirely forms a tubular con-

tainer 4, into which a specimen can be placed. Coatings 2 of Ab_v are located near the bottom of the container 4, and are concentrated in two locations.

The extended solid phase is composed of any material onto which the desired Ab_v can be effectively bound. For covalent binding with Ab protein, the solid phase material can be chosen to contain a functional carboxyl surface, with use of a water-soluble carbodiimide as a conjugation reagent. A preferred material is acrylic resin, which has a carboxylated surface that enables binding the desired Ab_v by conjugation. For materials with amino surface groups, reactive carboxyl intermediates can be prepared by reacting with succinic anhydride. A variety of inorganic supports, typically glass, can also be prepared for covalent coupling with Ab_v. Reference is made, for example, to "Enzymology, A Series of Textbooks and Monographs," Vol. 1, Chapter 1, 1975, the disclosure of which is incorporated herein by reference.

It is necessary to choose extended solid phase materials which bind Ab_v without causing serious interference with the assay steps. Hydrophobic polystyrene latex, for example, tends to stick non-specifically to many surfaces and molecules, and would not be a proper choice to carry specific immunoreagents. It can, however, be used as a deleting element in an affinity column for pretreatment of the specimen to remove undesired elements such as rheumatoid factor, etc. Hydrophilic polymeric latex, on the other hand, does not bind non-specifically and can provide functional groups to covalently conjugate the Ab protein.

The presence of non-specific agglutinators in a tissue specimen, particularly those coupled to immunoglobulins, can result in error by causing the binding of mobile microspheres to the extended solid phase even in the absence of specific Ag. Repeated washes during the assay would reduce the non-specific binding, but removal of the non-specific agglutinators is necessary in order to avoid such undesired binding. A simple polystyrene latex surface, for example, can passively delete some of the agglutinators, whereas an Ig G-coated surface provides a better affinity.

For convenience in the following description, the extended solid phase generally will be referred to as the preferred dipstick, although other forms may be used as explained above.

A typical viral particle has an envelope of many, usually over one hundred, identical Ag proteins or protein sets. The proteins provide very strong binding with specific Ab and form multiple conjugates or immune complexes. Highly specific Ab in monoclonal form has become available, either produced by hybridoma for the selected monoclonal mouse Ab, or by the human B-lymphocytes transformed by the Epstein-Barr virus for the human IgM. When properly chosen, these monoclonal antibodies can provide consistant and reproducible binding with virions. With a proper supply of specific Ab, the present direct binding immunoassay, in contradistinction with competitive binding immunoassay practiced in radioimmunoassay, can be a reliable and very rapid procedure since the incubation time for a kinematic equilibrium needed in competitive binding assays is not presently required.

In accordance with the method of the present invention, antiviral antibody Ab_v, either from the usual Ig fraction of the antisera or from monoclonal antibodies, is conjugated respectively with a solid phase dip stick as well as with a mobile solid phase, or the so called "monodispersed", microspheres. The functions of the dip stick are for the handling and the separation of bound from free antigens, whereas that of the mobile microspheres are for the detection of the formed immuno-complexes. Coupling techniques between the Ab protein and various solid phase materials are well developed (see, for example, the above-mentioned W.J. Dreyer, U.S. Patent 3,853,987).

The method of the present invention results in the following coupling:

Dip Stick + Ab_v + Viral Ag + Ab_v + Microsphere.

The amount of Ab required for covalent binding, however, can be a thousand times that of passive absorption to a plastic such as polyvinyl chloride and the economics of using such an amount of highly specific Ab_v can be prohibitive. An alternative way, retaining some strength of the covalent binding as well as the specificity of Ab_v, is to bridge the Ab_v and the solid phase with an antispecies antibody Ab_s, targeted against the F_c portion of immunoglobulin Ab_v. The F_c portion is shown in Figure 3 on page 9 of "Immunology" (1981), The Upjohn Company, Kalamazoo, Michigan. That is, inexpensive Ab_s, is first covalently bound to the solid phase, and the bound Ab_s attracts the species-specific F_c portion of Ab_v, leaving the functional epitope of Ab_v unaltered with regard to the viral Ag. Bridged with Ab_s, the immunoassay of the present invention brings about the following coupling:

Dip Stick + Ab_s + Ab_v + Viral Ag + Ab_v + Ab_s + Microsphere.

In the direct binding assay of the present invention, the couplings between the dipstick and Ab_v as well as the Microspheres and Ab_v are prepared in advance, and elements of non-specific agglutination in the fluid specimen are removed or deactivated for pretreatment prior to the direct

binding assaying as mentioned above. The assaying procedure of the invention is therefore simplified to the following steps:

(1) Insert the dipstick into the pretreated specimen.

(2) Wash.

(3) Insert the dipstick into the Ab_v coated microsphere dispersion.

(4) Wash.

(5) Detect the microspheres on the dipstick.

In order to use a minimal amount of wet chemistry, the present detection of attached microspheres on a dip stick is made independent of the immune chemistry. By concentration of the Ab_v at one end of the dipstick, the bound microspheres are concentrated at one location, which simplifies detection. The microspheres can include dye or fluorescent compounds for direct visual observation, or have metal elements or iron oxide doped or entrapped within in order to provide X-ray fluorescent or electromagnetic signals. Enzymatic amplification can also be designed into the microspheres, but it is not preferred in the present invention, because the enzymatic reaction must necessarily involve additional wet chemistry.

Conjugated with the microspheres, each Ag becomes coupled to, and therefore amplified with a solid phase material of 10^7 or more atoms for signal detection. With use of a good fluorescent microscope, the fluorescent microspheres can be seen at a size as small as 0.1 µm, clearly and without fading. In other words, the immune complexes can be counted individually as represented by the coupled mobile solid phase. Without using a microscope, a concentration of about one thousand fluorescent microspheres can be seen at the tip of the dipstick by the observer's unaided eyes under a UV lamp and with a dark field background. Colored microspheres of the same small size (0.1 µm) would require a few thousands in order to be directly visible. It is notable that in an infectivity assay or radioimmunoassay, the limiting sensitivity is about 10^6 virions or Ag, which is several orders of magnitude less sensitive than the above indicated sensitivity of the present assay. Moreover, in the present assay no detection instrument or additional wet chemistry is required for the indicated visual sensitivity.

Detection by X-ray fluorescence or by magnetic force enables minimal handling and avoids subjective judgement. In the above-mentioned copending U.S. patent Applications 313,711 and 331,859, the doping of metal elements in microspheres and methods of their detection are disclosed, and can be employed for the present detection step.

Magnetic microspheres can be made in polyglutaraldehyde material. The iron oxide entrapped particles should be demagnetized in order to have the Ab protein properly coated and their size selected without interference from spontaneous agglutination. A membrane filter with well defined pore size can eliminate, and thereby select the desired particle sizes without centrifuging. In the method of the present invention, the detection of magnetic microspheres is done similarly to that of a Faraday magnetometer where an intense magnetic field changes the weight of the magnetic particles.

As shown in Fig. 4, the dipstick for this embodiment has a non-magnetic sphere or ball 5 for assaying. The ball 5 is first conjugated with Ab_v and in the assay would be coated with the mobile, Ab_v coupled magnetic particles 8 in the presence of specific Ag. Before the assay, ball 5 is fitted into one of two sockets 6 connected by bridge member 7. After assaying, non-magnetic ball 5 is connected by bridge 7 to a second ball 9 of the same size, which is magnetic and is inserted into the other socket 6. The two balls and the bridge 7 form a dumb bell configuration, and would vibrate if either end is forced to oscillate, particularly if the oscillating frequency strikes a resonance with the natural frequency of the dumb bell. As illustrated in Fig. 5, operating like the needle of a record player, the dumb bell's assayed ball 5 may be coated with magnetic microspheres 8 is placed with a driving coil 10 whereas the second, highly magnetic ball 9 is enclosed in a pickup coil 11. The pickup coil 11 emits signals to be amplified by an audio amplifier 12 for display in meter 13. A high mu shield 14 separates the two balls. It is known that a sensitive vibrating reed synchronized by a stroboscope can detect an iron particle at about one µm in size without using superconducting designs (H. Zijlstra, Rev. Sci. Instruments 41, 1241; 1970). A vibrating reed coupled with an unknown amount of magnetic material cannot aim to vibrate at a set resonant frequency, but in the present dumb bell embodiment, the masses of the two balls remain essentially constant, as therefore also does the dumb bell's natural frequency. Accelerating at near the resonant frequency by a driving coil coupled to the assayed sphere, this very simple system can measure an iron conjugation at about 10 µm in total size. Such an amount of magnetic material is minimally required for one "bit" of signal in a sensitive magnetic tape. Using magnetic particles at about 0.4 µm each, a viral sensitivity of 10^3 -10^4 virions appears possible in the present embodiment.

Preferably, the present method employs a direct binding assay instead of a competitive binding assay where a dynamic equilibrium necessitates lengthy incubation. the disclosed method can, of course, be employed in a competitive protein binding assay as well. The roles of the immune an-

alytes Ab and Ag can also be interchanged, still making use of the immobilized solid phase for the signal amplification. Binding of Ab or various Ag molecules to the solid phase matter is well known, in passive absorption as well as in covalent coupling.

In the immunoassay of the present invention, the viral Ag, which appears in high multiplicity, is used as a bridge to connect the mobile and the immobilized solid phases. This connection can obviously be served by various other Ag with multiple Ab binding sites. In cases of certain Ag without repetitive binding sites which cannot specifically connect more than one monoclonal Ab, polyvalent Ab must be used instead.

With use of detection equipment providing multiple signal channels, such as the X-ray lines where each may correspond to the X-ray fluorescence of a particular metal element, the method of the invention can also be designed to assay several analytes in a single procedure where each analyte is represented by a particular element corresponding to a particular X-ray line.

Detection of different types of viruses can be done in accordance with the invention by conjugating a plurality of different Ab_v proteins capable of forming complexes with corresponding antigens of different viruses, respectively to the extended solid phase and to the mobile solid phase. The visual observation or other detection of any bound microspheres following the assay indicates that one or more of the different viruses is present in the specimen, and this assay, if positive, can be followed by assays for individual viruses of the different ones which were tested for simultaneously.

In another embodiment, the different viruses can be both simultaneously and individually detected. For such a test, the different Ab_v proteins corresponding to the antigens of a plurality of different types of viruses are conjugated to microspheres which are correspondingly labelled with different metal elements. When more than one type of the differently labelled microspheres are bound to the extended solid phase in the assay of the invention, they may be separately and simultaneously detected by X-ray fluorescence of the different metal element labels. In this way, the presence of corresponding individual types of viruses in the specimen are simultaneously and separately detected.

The extended solid phase and the dispersed microspheres which are conjugated with Ab_v, prepared as described above as individual components useful for the assay method of the invention, can be provided in the form of a virus detection kit comprising such components. Different kits may be provided, which differ as to the Ab_v coatings, and thus as to the viruses to the detected.

Such a kit may further include as an individual component, a latex solid phase for removing nonspecific agglutinators from a specimen prior to the assay. The preferred latex for this purpose is polystyrene coated with gamma immunoglobulin.

The extended solid phase and mobile solid phase components of the kit of the invention may be provided with Ab_v bound to Ab_s as disclosed above. Also as disclosed above, the microsphere component may be labelled, and the extended solid phase can take the form of part or all of a dipstick, syringe, tube or container, coated with Ab_v in at least one location, as disclosed.

Furthermore, the extended solid phase component may be provided with a plurality of different Ab_vproteins capable of forming complexes with corresponding antigens of different types of viruses. When it is so provided, the individual mobile solid phase component can be provided either to have the same plurality of Ab_v conjugated to each of the microspheres thereof, or a mixture of different types of microspheres can be provided, each type having conjugated thereto a different Ab_v protein of said plurality; or in a further variation, the mobile solid phase component can be provided in the form of separate batches of microspheres, each batch having conjugated thereto a different Ab_v protein of said plurality.

Viral Ag to be assayed can be the Herpes Simplex Viruses in various organs, particularly from the cervical PAP smears, from glycoprotein in cerebrospinal fluid; the Cytomegaloviruses (CMV) in urine, kidney, lung and brain; the Varicella-Zoster viruses in the brain; the Cox-Sackie B group viruses in the heart; the Measles viruses in lymph node and lung; the Respiratory syncytial viruses in nasal secretions and in the lung; the Hepatitis B viruses in serum; the Hepatitis A viruses in stool, etc. Since the clinical goal of viral assay is often the absence of the analyte of interest, and not its amount, several viral analytes can therefore be combined into a single test. For example, from a lung tissue, the Ab conjugated microspheres can include the tests for the Herpes viruses, the Cytomegaloviruses, and the Respiratory syncytial viruses. Only in the presence of a positive result, should the test proceed further for a specific and quantitative identification.

In the following description, materials and reagents were mostly obtained from Polysciences, Warrington, PA 18976. Membrane filters and cartridge holders were obtained from Bio-Rad Labs., Richmond, CA 84804.

EXAMPLE I

Coupling of Ab Protein to the Solid Phase Matter

Polymethyl methacrylate latex (Nature 249, 81; 1974) is used to from monodispersed microspheres with a functional carboxylated surface, and the conjugation reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, or ECDI, (latex microspheres and ECDI from Polysciences; see "methods in Enzymology", 70, 151; 1980) readily couples the carboxylated surface with the amino group of the protein, usually the lysyl or the alanyl residues:

(i) 10 µl of 0.09 µm carboxylated beads (5 x 10^{11} beads) are added to 100 µl of saline,

(ii) to 10 µl of the bead-saline suspension, add 90 µl of Ab_s (Rabbit anti-Goat) at the highest concentration available,

(iii) ECDI at 10mg/100 µl cold saline (4°C) is prepared and immediately mixed with the bead-saline-Ab suspension and the reaction is permitted to continue at 4°C for one hour with mild agitation of the mixture all the while, and with the pH kept at between 7.4 - 7.9 all the time,

(iv) coated beads are recovered with a membrane filter (Unipore from Bio-Rad) after washes and suspended in 10 ml saline (5 x 10^{10} beads/ml),

(v) 10 µl of Goat Ab_v (anti-cytomegalovirus, Polysciences) is added to the Ab_s coated beads at 4°C and mildly agitated for one hour. The coated beads are again washed and recovered with a filter, and suspended in 10 ml saline. The beads are ready for assay.

A dipstick of the same material as above for the microspheres is coated at the tip with the same antibodies as above.

EXAMPLE 2

Removal of Non-Specific Agglutinator

10 ml polystrene tubes are incubated with 2.5 ml of 0.1% glutaraldehyde in 0.1 M carbonate buffer, pH 9.0 for 3 hours at an elevated temperature (50-60°C). The tubes are cooled and washed thoroughly, and 2 ml of human IgG at 100µg/ml are added and incubated overnight at 4°C. The tubes are washed with phosphate-buffered saline (PBS) at pH 7.2 and the bound IgG is fixed by 2.5 ml of 0.05% glutaraldehyde in PBS for 1 hour at 25°C. Finally, the tubes are washed thoroughly and with the residue free aldehyde inactivated by 3 ml of 1% glycine in PBS. About 1.5% of the starting IgG remains bound to the tube surface.

A tissue specimen is lysed and homogenized to expose the virions and is incubated in the coated-tube for 1 hour at 25°C in order to remove the undesired agglutinators. Incubating in a second tube is done when necessary to thoroughly remove the agglutinators. The coated-tubes should not be reused for sensitive assays where the specimen contamination may be of concern.

EXAMPLE 3

Demagnetization of Micromagnetic Spheres

Iron containing microspheres are made of polyglutaraldehyde matrix (J. of Immunological Methods, 28, 341; 1979, and Polysciences.) The particles, however, are not provided with uniform sizes, and they tend to agglutinate spontaneously. The magnetic agglutination cannot be separated by simple sonification (ultrasound vibration). Prior to the coating of Ab, the magnetic microspheres must be demagnetized and their sizes sorted (Unipore filter from Bio-Rad.) During various stages of coating, storage, and assaying, the spheres must avoid the presence of magnetic field or magnetized material. Demagnetizing of the microspheres consists of the following steps:

The iron containing microspheres are placed in a gel, or in a frozen state to remain motionless. An oscillating magnetic field, usually with the household frequency of 60 Hz, is applied to the gel to magnetize the particles to the maximum, reversing back and forth at 60 Hz, and the field strength is reduced by spatial distance to a zero level as the gel physically leaves the field. The gel is sonicated at 10^5 Hz while returning to a liquid state and is placed in a high µ shield and in a gel state for storage in order to avoid the undesired spontaneous agglutination.

EXAMPLE 4

Display of Magnetic Signals

A sphere of non-magnetic material having a size of 40 µm is first costed with the desired Ab_v. This sphere is fitted into one of the two sockets of a bridge as shown in Fig. 4. The socket without a sphere is to be filled by inserting a highly magnetic sphere, also having a size of 40 µm, after the immunoassay. The second sphere does not participate in assaying procedures, and its sole function is to enhance the presence of the immunologically coated magnetic material of the first sphere.

The non-magnetic sphere, coated with Ab_v and held by the bridge, may fish out the Ab_v coated magnetic microspheres in the presence of specific Ag and become slightly magnetic. The second

highly magnetic sphere is inserted into the bridge after the immunoassay. A driving alternating magnetic force at a frequency near the resonant frequency of the dumb bell configuration is tuned at the first coated sphere, whereas a pickup coil surrounding the second magnetic sphere senses its vibration, which is amplified and displayed on a meter.

EXAMPLE 5

Assaying Procedure

The dipstick and microspheres prepared as in Example 1 and 2 are used.

In Example 1 the tip of the dipstick and the mobile fluorescent microspheres are each coated with certain Ab_v using Ab_s as bridging element, and in Example 2, non-specific agglutinators, such as the IgM class of rheumatoid factors, are removed from the serum specimen. The assaying procedure for CMV is simplified to the following steps.

A dipstick of anti-CMV Ab coated at its surface tip is inserted into a 0.5 ml serum specimen treated for the removal of non-specific agglutinators, in a sodium phosphate buffer, pH 7.0, for 10 minutes at 4°C with mild agitation. The dip stick is washed twice and inserted into 0.5 ml of anti-CMV Ab coated microspheres with 1/1000 dilution, for 5 minutes at 4°C with mild agitation, and again washed twice. The presence of fluorescent microspheres at the coated area, the tip, of the dipstick is detected by examining visually with UV illumination against a dark field background. As few as several thousand CMV virions in 0.5 ml serum are detected in this procedure.

Claims

1. A method for detection of target proteins or viruses in a specimen, characterized in that said specimen, treated to remove undesired components, is contacted with an extended solid phase support having conjugated thereon antitarget antibody (Ab_v) to form immuno-complexes with antigens characteristic of the proteins or viruses to be detected;
the extended solid phase is separated from the specimen;
said separated extended solid phase is contacted with a mobile solid phase consisting of dispersed microspheres having conjugated thereto said Ab_v to bind said microspheres to said immuno-complexes;
the extended solid phase is separated from said mobile solid phase; and
the presence of microspheres bound to said extended solid phase is detected, whereby the presence of proteins or viruses in said specimen is detected or determined.

2. A method according to claim 1, characterized in that antispecies antibody (Ab_s) is respectively covalently bound to the extended solid phase and to the mobile solid phase, and Ab_v which forms an immuno-complex with said Ab_s is coupled therewith, whereby there is conjugated respectively to said extended solid phase and to said mobile solid phase Ab_v which is capable of forming immuno-complexes with antigens of proteins or viruses to be detected, and preferably the Ab_v is monoclonal.

3. A method according to claim 1 or 2, characterized in that the microspheres are labelled for detection with (i) dyes or fluorescent compounds, (ii) magnetic materials or (iii) metal elements, and are detected as follows:
when labelled as in (i), the labelled microspheres bound to the immuno-complexes are visually detected directly or with a microscope; when labelled as in (ii), the microspheres are detected electonically; and when labelled as in (iii) the labelled microspheres bound to the immuno-complexes are detected by X-ray fluorescence, with (iii) being preferred.

4. A method according to any one of the preceding claims, characterized in that a plurality of different Ab_vproteins capable of forming complexes with corresponding antigens of different types of proteins or viruses are respectively conjugated to the extended solid phase and to the mobile solid phase whereby the presence of one or more of a plurality of different types of proteins or viruses in the specimen is detected.

5. A method according to claim 4, characterized in that the different Ab_v proteins conjugated to the mobile solid phase are conjugated to microspheres which are correspondingly labelled with different metal elements, thereby forming a plurality of differently labelled types of microspheres, said differently labelled types of microspheres are bound to immuno-complexes of corresponding protein or viral antigens on the extended solid phase, said immuno-complexes having been formed respectively from the plurality of different Ab_vproteins conjugated to the extended solid phase and corresponding antigens of different types of proteins or viruses present in the specimen and the differently labelled types of microspheres are separately detected by X-ray fluorescence of the different metal element labels thereby simultaneously and separately determining the presence of corresponding individual types of proteins or viruses in the specimen.

6. A kit for detection of target proteins or viruses, characterized in that it comprises as individual components:

(a) an extended solid phase having conjugated thereon antitarget antibody (Ab_v) capable of forming immunocomplexes with antigens characteristic of the proteins or viruses to be detected; and

(b) a mobile solid phase consisting of dispersed microspheres having said Ab_v conjugated thereto, and optionally further including as an individual component:

(c) a latex solid phase capable of removing non-specific agglutinators from a specimen, said latex preferably being polystyrene coated with gamma immuno-globulin.

7. A kit according to claim 6, characterized in that the extended solid phase component (a) and the mobile solid phase component (b) respectively have antispecies antibody (Ab_s) covalently bound thereto, and Ab_v which forms an immunocomplex with said Ab_s coupled therewith, whereby said extended solid phase component (a) and said mobile solid phase component (b) respectively have conjugated thereto Ab_v which is capable of forming immuno-complexes with antigens of proteins or viruses to be detected.

8. A kit according to claim 6 or 7, characterized in that the microspheres of component (b) are labelled for detection with dyes, fluorescent compounds, magnetic materials or metal elements.

9. A kit according to any one of claims 6 to 8, characterized in that the extended solid phase component (a) forms at least part of a dipstick, syringe, tube or container, and preferably the extended solid phase forms at least part of a dipstick, said extended phase being included at least at an end of said dipstick.

10. A kit according to any one of claims 6 to 9, characterized in that the extended solid phase component (a) has conjugated thereto a plurality of different Ab_v proteins capable of forming complexes with corresponding antigens of different types of proteins or viruses, and either:

(1) the microspheres of mobile solid phase component (b) each has conjugated thereto a plurality of said different Ab_v proteins; or

(2) the mobile solid phase component (b) consists of a mixture of different types of microspheres, each type having conjugated thereto a different Ab_v protein, the different Ab_vproteins being capable of forming complexes with corresponding antigens of different proteins or viruses, or

(3) the mobile solid phase component (b) consists of separate batches of microspheres, each batch having conjugated thereto a different Ab_v protein, said different Ab_v proteins being capable of forming complexes with corresponding antigens of different proteins or viruses.

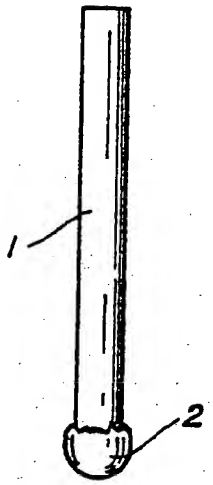

FIG. 1

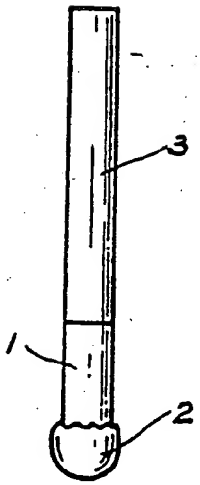

FIG. 2

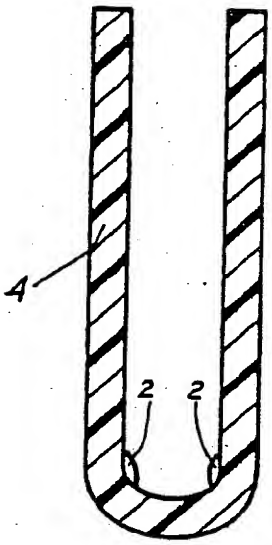

FIG. 3

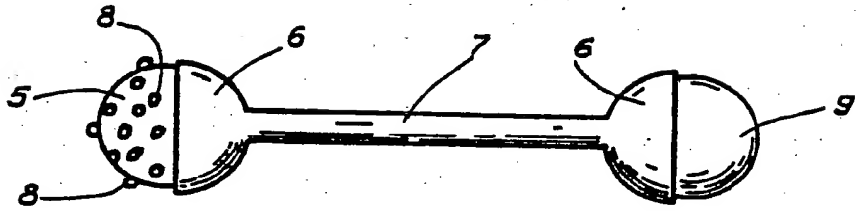

FIG. 4

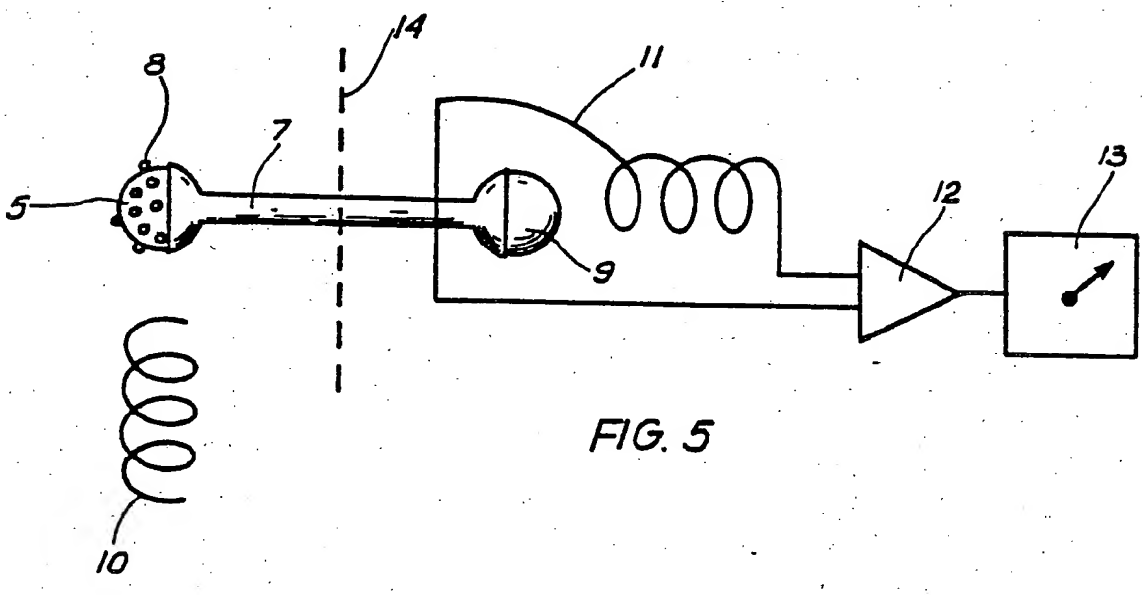

FIG. 5

European Patent Office

EUROPEAN SEARCH REPORT

Application number

EP 86 11 5797

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	JOURNAL OF CHROMATOGRAPHY - BIOMEDICAL APPLICATIONS, vol. 376, 1986, pages 175-189, Amsterdam, NL; C.J. GRIBNAU et al.: "Particle-labelled immunoassays: A review" * page 179, paragraph 3 - page 180, figure 2 *	1-3,6	G 01 N 33/68 G 01 N 33/569 G 01 N 33/543
A	WO-A-8 200 203 (INSTITUT PASTEUR) * abstract *	1	
A	EP-A-0 077 671 (ORTHO DIAGNOSTIC SYSTEMS INC.) * claims; figure 5 *	1	
A	EP-A-0 155 224 (CHROMAGEMICS) * abstract *	1	TECHNICAL FIELDS SEARCHED (Int. Cl.4) G 01 N 33/00
A,D	US-A-3 853 987 (W.J. DREYER) * complete *	1	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	08-07-1987	GREEN C.H.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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